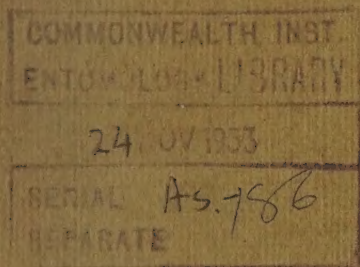


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A NEW SEX-LINKED MUTATION IN THE SILKWORM, BOMBYX MORI L¹.

Yoshimaro TANAKA

As I have described in my previous papers (1917, 1922) there had been found a case of sex-linkage in the silkworm with regard to inheritance of the translucent skin character. In 1918, on the other hand, I happened to find a number of abnormal larvae in a hybrid strain reared in my silkworm-house. In the subsequent years, the hereditary behaviour of this mutant character, the "elongate" as I called it, has been thoroughly studied, and now it has become evident that the mutation in question is linked to sex, and forms a second case of sex-linkage in the silkworm. Another interesting point in this connection is the occasional appearance of exceptional males in the course of the experiments. At first, I supposed it to be a similar case to the non-disjunction of BRIDGES (1916); the later experiments have, however, revealed that it was due to mere somatic variation, but not due to any genetic irregularities.

I. CHARACTERISTICS OF THE "ELONGATE"

Larva (Plate 1, Fig. 1). First and second abdominal segments are rendered markedly long, and the skin is longitudinally stretched, resulting in the disappearance of the intersegmental folds in this region. That the body is much softer to the touch than in the normal larva is another characteristic of the mutant.

Pupa (Plate 1, Figs. 2 and 3). The Characteristic of the mutation is most conspicuously developed in the pupal stage. The brown coloured primary cuticula is torn in the boundaries of the first and second, second and third, and often also of the third and fourth abdominal segments, disclosing the colourless secondary or inner cuticula as two or three narrow white bands in those parts. The second segment, which is normally the smallest of the pupal abdominal segments, is rendered more or less larger, sometimes bigger than any of other abdominal

¹ Contributions from the Sericultural Laboratory, Kyushu Imperial University, No. 1.

segments. In such extreme cases as shown in Fig. 2, to the right, and in Fig. 3, the middle of the lower row, the segment in question attained a tremendous size, making the animal look like a humpback.

Moth. The first abdominal segment is longer than in the normal.

Cocoon. The cocoons spun by the elongate larvae are often deformed and are less hard and firm than those spun by the normals.

The mentioned characteristics of the mutant are, as a rule, distinct and easily distinguishable from the normal type.

2. ORIGIN OF THE "ELONGATE"

In a hybrid family, No. 18*amb*, reared in the spring of 1918, a number of abnormal larvae attracted my attention, in which the anterior segments were markedly long in comparison with the posterior ones. When picked up with the fingers, they contracted the posterior body, but not the anterior, while the normals contracted the whole body, thus making the peculiarity even more striking. When killed, the peculiarity usually became less marked. Such abnormal caterpillars, later called "elongates," were separated and counted. The result follows:

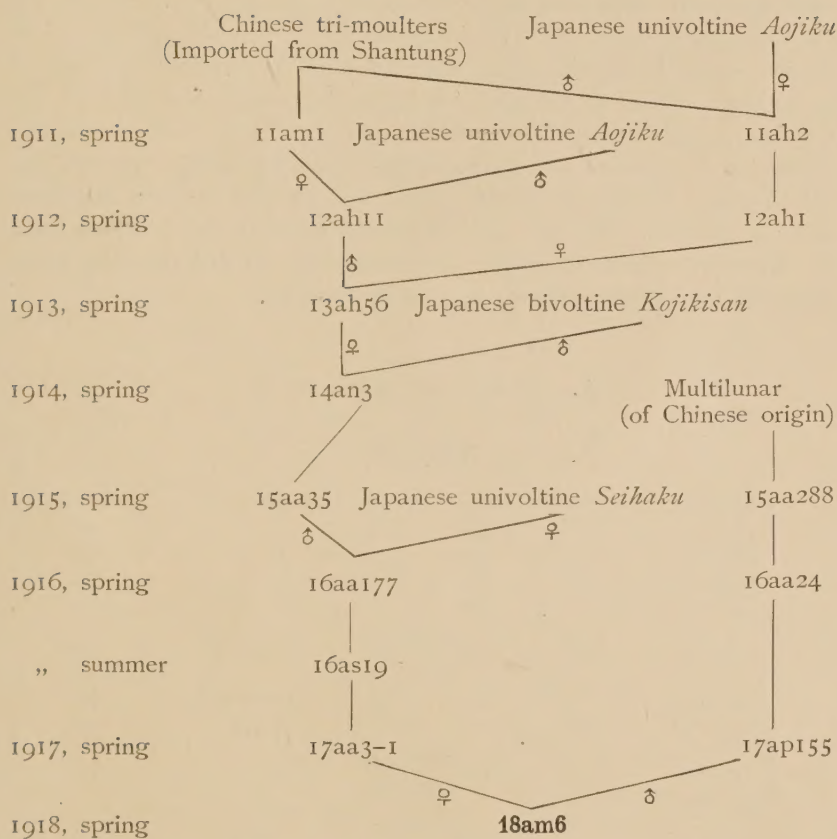
Table 1

		Tri-moulters	Tetra-moulters	Totals
Normal	{ Plain, knobbed, white	57	75	132
	{ Plain, smooth, white	46	63	109
Elongate	{ Plain, knobbed, white	17	26	43
	{ Plain, smooth, white	15	19	34

That is 241 normals to 77 elongates, 318 in all. The sexes were not discriminated in the larval stage, but there is a strong reason for the supposition that the 77 abnormalities were all female, because all elongate individuals, 21 in number, allowed to develop into moths were female.

The pedigree of this family is given below.

Table 2



The female parent of the family 18am6 was plain, smooth, white blooded, while the male parent was plain, knobbed, white-blooded.

As is evident in the pedigree, the female parent originates from a Chinese tri-moulting race to which Japanese races (*Aojiku*, *Kojikisan* and *Seihaku*) were crossed in three different generations, while the male parent is a pure breed of Chinese origin. All these strains had been cultured under my own observation for several (at least four) generations, and in no case had so many abnormalities (elongates) been found up to the date mentioned.

3. EXPERIMENTAL RESULTS

Since the elongate individuals were obtained, for the first time, in 1918, they and their offspring formed the sole source of the elongates in the following experiments. Their normal mates were taken from various strains reared in my silkworm-house for many generations and proved to be pure normal so far as the character in question is concerned, e.g. *Chiyozuru*, *Shirogasuri*, *Arayahime*, stony, black pupa, monster-producing, translucent etc., thus resulting in the segregation of markings, translucency, number of moults, pupal and cocoon colours, and some other characters. For the sake of simplicity, however, I will mention the segregation of the elongate *versus* the normal and the sexes only, disregarding all other characters for the present.

A. Elongate ♀ × Normal ♂

a) F₁

Table 3

Family No.	Normals	Elongates	Totals
19am6-1	398	0	398
19am6-2	340	0	340
19am6-3	322	0	322
20bm6-3	372	0	372
22am6-9	267 { ♀133 ♂134	0	267
22am6-10	349	10 (all ♂♂)	359
22am6-11	247	1 (♂)	248
22cm6-2	122 { ♀55 ♂67	0	122
22cm6-3	129 { ♀78 ♂51	2 (♂♂)	131
22cm6-4	179 { ♀81 ♂98	0	179
22cm6-23	124 { ♀40 ♂84	1 (♂)	125
22cm6-26	106 { ♀56 ♂50	0	106
22cm6-27	97 { ♀45 ♂52	0	97
Totals	3052	14	3066
Expected	3066	0	

Summing up the families in which the sexes were distinguished, the result follows :

	Normals		Elongates		Totals
	♀	♂	♀	♂	
Experimental	488	536	0	14	1038
Theoretical	519	519	0	0	

b) F

Table 4

Family No.	Normals	Elongates	Totals
19bm6-2	232 (♀+♂)	67 (17 moths emerged were all ♀♀)	233
19bm6-3	184	49	233
19bm6-4	203	31	234
20cm6-1	171 { ♀ 67 ♂ 104	46 { ♀ 44 ♂ 2	217
20cm6-2	278 { ♀ 86 ♂ 192	63 { ♀ 63 ♂ 0	341
22bm6-3	222 { ♀ 75 ♂ 147	89 { ♀ 89 ♂ 0	311
22bm6-4	193 { ♀ 6 ♂ 126	57 { ♀ 57 ♂ 0	250
22bm6-5	151 { ♀ 40 ♂ 111	38 { ♀ 38 ♂ 0	189
22bm6-6	124 { ♀ 43 ♂ 81	37 { ♀ 37 ♂ 0	161
22bm6-7	113 { ♀ 3 ♂ 80	29 { ♀ 29 ♂ 0	142
22cm6-21-1	133 { ♀ 49 ♂ 84	30 { ♀ 30 ♂ 0	163
22cm6-21-2	83 { ♀ 31 ♂ 52	29 { ♀ 29 ♂ 0	112
22cm6-21-3	206 { ♀ 71 ♂ 135	61 { ♀ 60 ♂ 1	267
22ch20	70 { ♀ 22 ♂ 48	21 { ♀ 21 ♂ 0	91
Totals	2363	647	3010
Expected	2257.5	752.5	
Standard error	±23.75	±23.75	

Summing up the families in which sexes were discriminated, the result follows :

	Normals 1744		Elongates 500		Totals 2244
	♀	♂	♀	♂	
Experimental	584	1160	497	3	
Theoretical	561	1122	561	0	
Standard error	± 20.55	± 23.69	± 20.55		

c) F_3

- 1) F_2 normals mated to each other gave the following result :

Table 5

Family No.	Normals	Elongates	Totals
19cm6-3	135	31	166
20am6-2	404	120	524
Totals	539	151	690

In these families, the sexes were not distinguished during the larval life. The elongate moths that emerged from No. 20am6-2, however, were all female, hence we can suppose that the same segregation as in F_2 occurred in both of the families :

- 2) The elongate females from F_2 generation were mated by their normal brothers, the result follows.

Table 6

Family No.	Normals	Elongates	Totals
21am6-5	190 { ♀ 95	228 { ♀ 118	418
	♂ 95		
21am6-6	112 { ♀ 61	143 { ♀ 70	255
	♂ 51		
Totals	302 { ♀ 156	371 { ♀ 188	673
	♂ 146	♂ 183	

B. Normal ♀ × Elongate ♂

a) F₁

Table 7

Family No.	Normals	Elongates	Totals
22am6-12	156 (♂♂)	142 (all ♀♀)	298
22am6-13	188 { ♀ 2 ♂ 186	167 (all ♀♀)	355
22cm6-5	151 (all ♂♂)	140 (all ♀♀)	291
22cm6-6	60 (all ♂♂)	78 (all ♀♀)	138
22cm6-7	137 (all ♂♂)	125 (all ♀♀)	262
22cm6-22	21 (all ♂♂)	28 (all ♀♀)	49
22ch12-3	75 { ♀ 1 ♂ 74	62 (all ♀♀)	137
Totals	<div> <div>788</div> <div>♀ 3 ♂ 785</div> </div> <div> <div>742</div> <div>♀ 742 ♂ 0</div> </div>		1530
Expected	<div> <div>0</div> <div>765</div> </div> <div> <div>765</div> <div>0</div> </div>		
Standard error	±19.56 ±19.56		

b) F₂

Table 8

Family No.	Normals	Elongates	Totals
22bm5-8	133 { ♀ 80 ♂ 53	110 { ♀ 54 ♂ 56	243
22bm6-9	66 { ♀ 31 ♂ 35	30 { ♀ 16 ♂ 14	96
22bm6-10	90 { ♀ 66 ♂ 24	38 { ♀ 20 ♂ 18	128
22bm6-11	91 { ♀ 44 ♂ 47	68 { ♀ 36 ♂ 32	159
22bm6-12	88 { ♀ 62 ♂ 26	50 { ♀ 27 ♂ 23	138
Totals	<div> <div>468</div> <div>♀ 283 ♂ 185</div> </div> <div> <div>296</div> <div>♀ 153 ♂ 143</div> </div>		764
Expected	<div> <div>191</div> <div>191</div> </div> <div> <div>191</div> <div>191</div> </div>		
Standard error	±11.97 ±11.97 ±11.97 ±11.97		

C. Back Crossing

a) Pure normal ♀ × F₁ (normal ♀ × elongate ♂) normal ♂

Table 9

Family No.	Normals		Elongates		Totals
22bm6-15	219	♀ 74 ♂ 145	75	♀ 75 ♂ 0	294
22b 6-16	207	♀ 75 ♂ 132	51	♀ 51 ♂ 0	258
Totals	426		126		552
	♀	♂	♀	♂	
Expected	149	277	126	0	
Standard error	±10.17	±11.91	±10.17	0	

b) F₁ (normal ♀ × elongate ♂) elongate ♀ × pure normal ♂

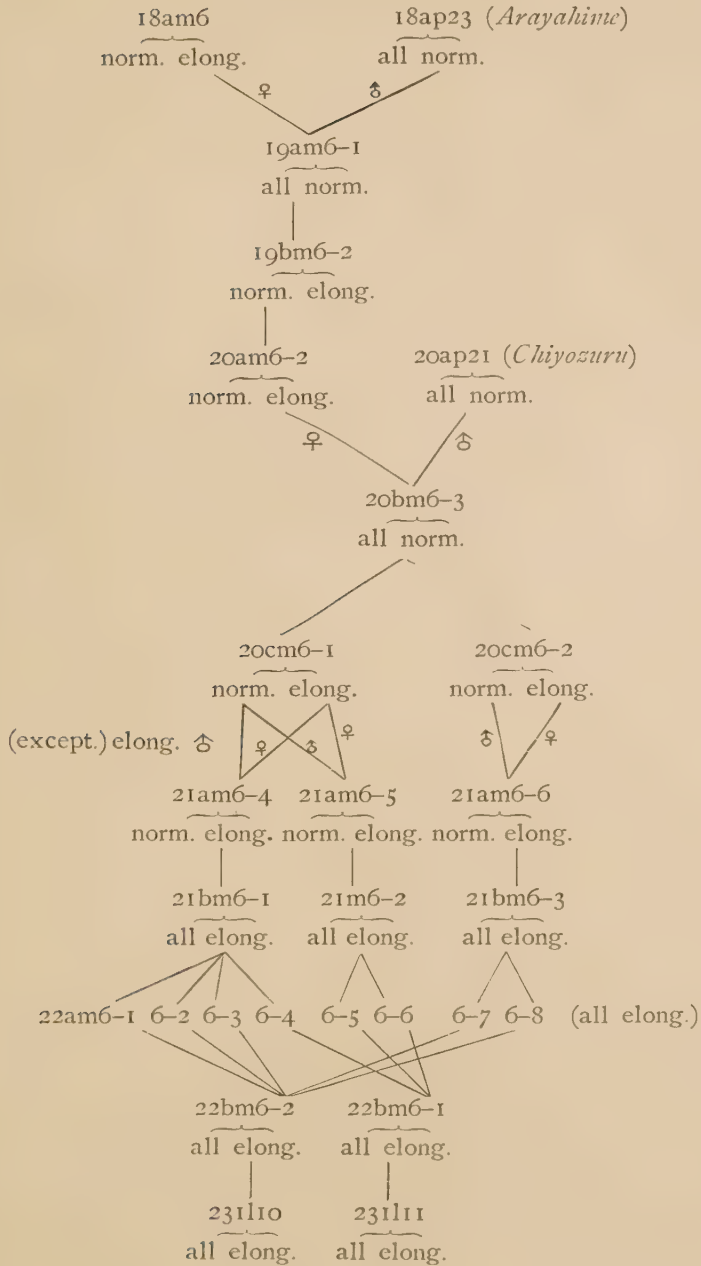
Table 10

Family No.	Normals		Elongates	Totals
22bm6-21	370	♀ 195 ♂ 175	0	370

D. Pure Elongate Strains

In the summer of 1921, mating was made between elongate males and females, the offspring, that were reared in the spring of 1922, consisted of the elongate only. There were eight families cultivated separately, in seven of which the number of individuals was counted as 2024. In regard to the two subsequent generations each generation was reared in two mixed lots, in which no exception was observed. In four families of these pure strains, sexes were discriminated before spinning, giving 568 females and 563 males, 1131 altogether. The pedigree of the pure elongate strains is given below.

Table II



E. Exceptional Individuals

As may be seen in the Tables, there were sometimes met with exceptional males and females in the course of experiments. Such cases are collected below.

Table 12

Family No.	Nature of the Family	Females (normal)	Males (elongate)
22am6-10	F ₁ from elong. ♀ × norm. ♂	—	10*
22am6-11	"	—	1
22cm6-3	"	—	2
22cm6-23	"	—	1
20cm6-1	F ₂ from elong. ♀ × norm. ♂	—	2*
22am6-13	F ₁ from norm. ♀ × elong. ♂	2	—
22ch12-3	"	1	—
Totals		3	16

* These individuals were used in the following experiments.

To examine the genetic character of the "exceptional" individuals, the following crossings have been made.

Pure normal ♀ × exceptional elongate ♂.

a) F₁

Table 13

Family No.	Normals	Elongates	Totals
22bm6-13	222 { ♀ 77 ♂ 145	91 { ♀ 91 ♂ 0	313
22bm6-14	124 { ♀ 40 ♂ 84	28 { ♀ 28 ♂ 0	152
22bm6-17	293 { ♀ 100 ♂ 193	68 { ♀ 68 ♂ 0	361
22bm6-22	215 { ♀ 72 ♂ 143	94 { ♀ 94 ♂ 0	309
22bm6-20	134 { ♀ 31 ♂ 103	33 { ♀ 33 ♂ 0	167
Totals	988 { ♀ 320 ♂ 668	314 { ♀ 314 ♂ 0	1302

b) F₂ (F₁ normal ♀ × F₁ normal ♂) from (a)

Table 14

Family No.	Normals	Elongates	Totals
22cm6-13-1	62 { ♀ 28 ♂ 34	0	62

Family No.	Normals	Elongates	Total
22cm6-13-2	41 { $\begin{smallmatrix} \text{♀} 18 \\ \text{♂} 23 \end{smallmatrix}$	0	41
Totals	103 { $\begin{smallmatrix} \text{♀} 46 \\ \text{♂} 57 \end{smallmatrix}$	0	103

c) F_2 (F_1 elongate ♀ \times F_1 normal ♂) from (a)

Table 15

Family No.	Normals	Elongates	Totals
22cm6-13-3	20 { $\begin{smallmatrix} \text{♀} 12 \\ \text{♂} 8 \end{smallmatrix}$	13 { $\begin{smallmatrix} \text{♀} 9 \\ \text{♂} 4 \end{smallmatrix}$	33
22cm6-13-4	40 { $\begin{smallmatrix} \text{♀} 19 \\ \text{♂} 21 \end{smallmatrix}$	32 { $\begin{smallmatrix} \text{♀} 15 \\ \text{♂} 17 \end{smallmatrix}$	72
Totals	60 { $\begin{smallmatrix} \text{♀} 31 \\ \text{♂} 29 \end{smallmatrix}$	45 { $\begin{smallmatrix} \text{♀} 24 \\ \text{♂} 21 \end{smallmatrix}$	105

d) Back-crossing (F_1 elongate ♀ \times pure normal ♂)

Table 16

Family No.	Normals	Elongates	Totals
22cm6-24	163 { $\begin{smallmatrix} \text{♀} 91 \\ \text{♂} 72 \end{smallmatrix}$	0	163
22cm6-25	110 { $\begin{smallmatrix} \text{♀} 55 \\ \text{♂} 55 \end{smallmatrix}$	0	110
Totals	273 { $\begin{smallmatrix} \text{♀} 146 \\ \text{♂} 127 \end{smallmatrix}$	0	273

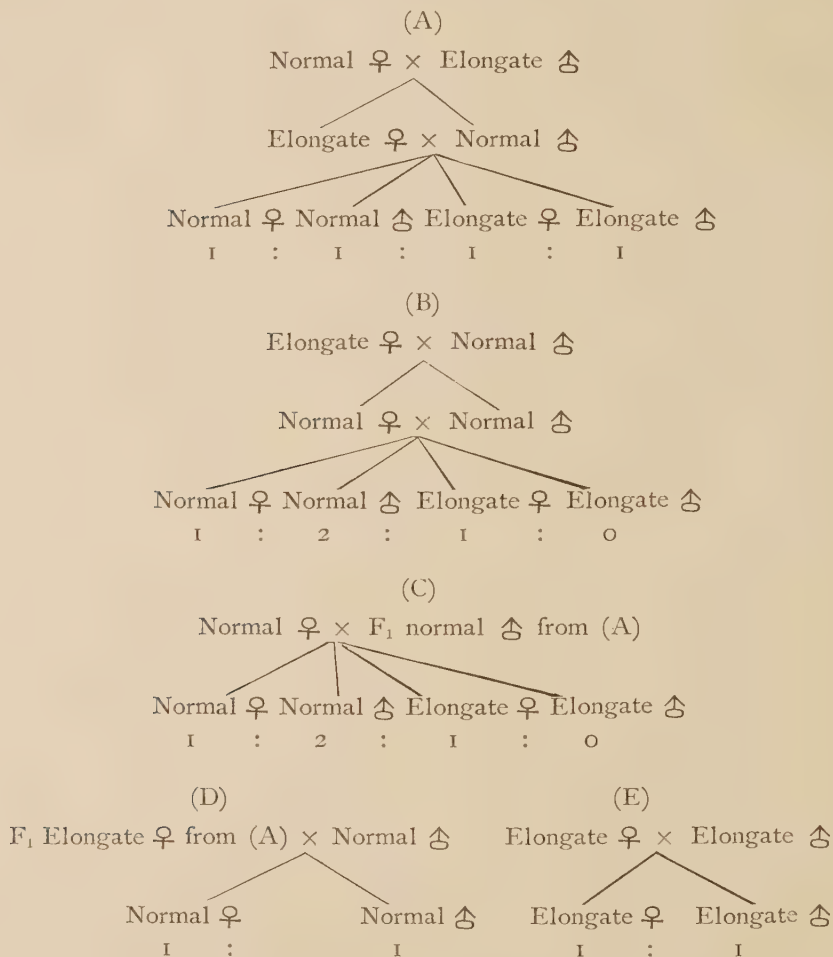
Pure elongate ♀ \times exceptional elongate ♂

Table 17

Family No	Normals	Elongates	Totals
21am6-4	184 { $\begin{smallmatrix} \text{♀} 95 \\ \text{♂} 89 \end{smallmatrix}$	149 { $\begin{smallmatrix} \text{♀} 72 \\ \text{♂} 77 \end{smallmatrix}$	333
22bm6-1	170 { $\begin{smallmatrix} \text{♀} 88 \\ \text{♂} 82 \end{smallmatrix}$	142 { $\begin{smallmatrix} \text{♀} 71 \\ \text{♂} 71 \end{smallmatrix}$	312
Totals	354 { $\begin{smallmatrix} \text{♀} 183 \\ \text{♂} 171 \end{smallmatrix}$	291 { $\begin{smallmatrix} \text{♀} 143 \\ \text{♂} 148 \end{smallmatrix}$	645

4. CONCLUSION

If the elongate is sex-linked in a similar way to the translucent of an Italian yellow race, the scheme of inheritance ought to be as follows :



As is shown in Tables 3-10, actual numbers are not very far from the theoretical ones calculated upon the above scheme ; the deviations do not exceed three times the standard errors, with the exception only of F₂ families from the crossing normal ♀ × elongate ♂, in which the deficiency of the elongate is somewhat striking. This fact may, perhaps, be explained by differential vitality of the normal and the elongate. It

is seen in the Tables referred to, that the actual numbers of the elongate come always behind the expectation, especially where the mortality is high¹⁾. An interesting case in this connection has been obtained after the Tables for this paper had been completed, and thus was not included in them. An F_2 family, *22dm6-4-3*, from the cross elongate ♀ × normal ♂, was reared during the period from October to December of 1922. This unseasonable culture was accomplished with considerable difficulty, especially on account of bad conditions of food. 308 larvae hatched on 13th of October, and they were observed and counted for the first time on 4th of December.

	Females	Males	Totals
Normals	65	126	191
Elongates	46	1	47
Totals	111	127	238

Up to that date, 77.3 per cent. still survived. It was about this time that the mulberry leaves begun to be frosted, and to turn yellow, hard and fibrous. After that the mortality increased rapidly, survival percentage dropping to 43.5 per cent. after only four days, or on 8th of the same month, as shown below :

	Females	Males	Totals
Normals	43	71	114
Elongates	19	1	20
Totals	62	72	134

From this result, it is evident that the elongates suffer under the influence of unfavourable conditions more heavily than the normals ; in the first count, the elongates occupied 19.7 per cent. of the entire family, while in the second the percentage came down to 14.9.

The only exception to the above statement is the case given in Table 6. There were 371 elongates to 302 normals while the expectation was equality in the numbers of the two types. Considering the rather high survival percentage in the families concerned, 84.6, the case might be regarded as accidental.

Now we come to the consideration of the "exceptional" individuals. From Tables 12-17, we learn that the "exceptional" males behave quite similarly to the *heterozygous normals* in inheritance, and they proved themselves not "exceptional" in a genetical sense. While externally they resemble the true elongate, and are easily distinguishable from

1) The initial number of the individuals in each family is not given in the Tables, but it is recorded in my protocols, from which we can calculate the mortality for each family.

the normal, a minute examination will enable us to make out some difference between the true elongate and the "exception": it is a general rule in the latter that the elongation of the segments is somewhat slighter and the body harder than in the true elongate type. Though no experiment has as yet been made with the exceptional normal *females*, it seems to me highly probable that they are also due to mere phenotypic aberration.

Admitting certain deviations due to the differential vitality, and counting the "exceptional" elongate males among the normals, and the "exceptional" normal females among the elongates, we come to the conclusion that the theoretical expectation is fairly realised, and the statement that *Bombyx mori* belongs to **ZZ ZW** type in its chromosomal constitution is once more proved from the experimental side.

If we denote the elongate gene by **e** and its normal allelomorph by **E**, both the genes **e** and **o^s** (sex-linked translucent), their normal allelomorphs **E** and **O^s** also, are located in the sex-chromosome, **Z**, consequently a certain form of linkage must take place in a dihybrid for the said two genes. This point will be made out on a future occasion.

The elongate silkworm has, so far as I am aware, never been reported elsewhere, and I am confident that its origin is due to a mutation which occurred in one of my cultures. The family *18am6* in which the elongate made its first appearance was, most probably, derived from the cross **Ee** ♀ × **Ee** ♂, because it gave apparently normal males, normal females and elongate females in 2 : 1 : 1 ratio. As the genetic constitution of the normal female is always **Ee**, the mutation can easily be supposed to have been introduced by the male parent which was taken from a pure breed of Chinese multilunar. Notwithstanding the recessive nature of the elongate, it could not, in virtue of its linkage with sex, pass many generations without being detected. The mutation must have occurred in the spring of 1917 or of 1916, but not earlier.

On the other hand, I have occasionally met with in various strains having no apparent blood-relation with those described in the foregoing pages occurrence of caterpillars externally similar to the elongate. The elongate character in them was seldom markedly developed, and they were usually small in number (one or two for example, when they appeared), but also, though rarely, in considerable numbers. Though whether they are genetically identical with the elongate has not yet been ascertained, I am not unable to make a provisional suggestion in this direction. The elongate-like larvae occurred in a pure breed of Japanese *bivoltine Aojiku* gave, when mated among themselves, not only the elongates but

also the normals. Even after a continued selection for four generations, the character did not *fix*, segregating both types in each generation. The percentage of abnormals did also not increase at all, it remained always under 25 per cent. It can be imagined, therefore, that they were quite different from *the elongate* in a genetical sense, if they have any, or were mere somatic aberrants.

I wish to express my thanks to Dr. T. KAGAYAMA, Director of Imperial Sericultural Experiment Station, for his kindness in permitting me to make part of the present experiments in Fukushima Branch Station for several years. My obligation is also due to Mr. S. MATSUNO, my assistant, who continued the experiments in a satisfactory manner during my two years' travelling abroad.

5. SUMMARY

1. A second case of the sex-linkage in the silkworm is reported in the present paper.
2. The character concerned is called "elongate."
3. The elongate is a new character first observed in the spring of 1918.
4. We denote the elongate gene by **e** and its normal allelomorph by **E**.

Sericultural Laboratory, Department of Agriculture,
Kyushu Imperial University. June 1923.

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EXPLANATION OF PLATE I

- Fig. 1. The larvae, full grown, natural size.
 From left to right:
 1. Elongate, dorsal.
 2. Normal, dorsal.
 3. Elongate, lateral.
 4. Normal, lateral.
- Fig. 2. The pupae, magnified.
 Left. Normal.
 Right. Elongate.
- Fig. 3. The pupae, natural size.
 Upper row. Normals.
 From left to right:
 1-3. Dorsal.
 4. Ventral.
 5. Lateral.
- Lower row. Elongates.
 From left to right:
 1-3. Dorsal, in the order of the degree of development of the characteristic.
 4. Ventral.
 5. Lateral.

La preskorektado de ĉi tiu verko de D-ro Tanaka preskaŭ finiĝis je la 1-a de Septembro, 1923, kiam okazis en Tokio la tertremego, kiun sekvis brulego. Nia presejo ankaŭ fariĝis viktimo de la ruiniĝo. Estas tre bedaŭrinde, ke la publikigo de la verko tiel prokrastis pro tia kaŭzo. Ni tie ĉi esprimas nian tutkoran apologion al D-ro Tanaka. Ankaŭ ni dankas al la presisto, kiu malgraŭ sia malfacila situacio penadis ripeti la laboron por ni tiel rapide.—*Redakcio.*

(La 18-an de Marto, 1924.)



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3



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